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Evaluation of Phytoconstituents and Mechanism of Action of *Hunteria umbellata* in the Management of Type II Diabetes Mellitus

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ABSTRACT: *Hunteria umbellata* (HU) has shown to be beneficial in health conditions including diabetes mellitus. This study explores fully and evaluates, the pharmacological and pharmacodynamics of HU in the treatment of diabetes mellitus. The study design is a double-blind randomised controlled trial (RCT) of forty-two subjects, 18 years and above with diabetes type II matched by age, sex and locality last Four weeks. The effect of the fractional components of HU on fasting blood glucose levels was undertaken daily for four weeks. The outcome of the two groups, following the standard principles for RCT, was compared. Statistical analysis was carried out using the SPSS (v27), while considering the mean difference, attrition rate and missing data in the analysis of variance procedure. The statistical uncertainties were expressed in 95% two-sided confidence intervals. A *p*-value of < 0.05 indicated statistical significance. The result indicates a significant impact on future patients as it has specified a pharmacological component that is effective, cheap, and readily available in the treatment of the disease with high prevalence and comorbidity.

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Many medicinal plants have been reported to protect or exhibit effective curative effects against a host of physiological diseases due to their phytochemical components which are effective antioxidants. Antioxidants have been hypothesized to have a protective effect against diabetes. This study evaluated the phytoconstituents of potent fraction of *Hunteria umbellata* seed and its mechanism of action as a potent antidiabetic plant. Healthy *H. umbellata* seeds were sun-dried and pulverized before being subjected to ethanolic extraction and partitioning. Both extract and fractions of Whole and Peeled seeds were screened for the presence and levels of phytochemicals. The extracts and fractions were studied for inhibition against α-amylase and α-glucosidase. Equal weight (150 mg/kg) of most active fraction (Whole Aqueous) and placebo were administered on Subjects on for 28 days. Measurements of biometric (age, blood pressure, fasting blood glucose, body mass index) and biochemical (total protein, ALT, AST, urea, creatinine, Trig, and HDL) parameters were taken before and after administration for the assessment of antidiabetic and toxicological effects of the Whole Aqueous fraction, respectively. The samples contained tannins, flavonoids, alkaloids, steroids, glycosides, terpenoids, resins, phenols and saponins in different proportions. Total phenolics was determined as a gallic acid equivalent, flavonoids as quercetin, tannins as catechin, and alkaloids as atropine. The average age of Subjects spread across different socioeconomic groups like farming, civil service, teaching, artisan, retirees, and clergy was 58 ± 0.8. The results showed

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that Whole Aqueous *H. umbellata* fraction have higher phenolic (17.16 ± 0.90) and flavonoids (4.31 ± 0.02) contents than the peeled. It also exhibited higher inhibitory effect on α-amylase (IC_{50}=0.134±0.028) and α-glucosidase (IC_{50}=0.247±0.001) which was not significantly different from the Unpeeled fraction. The blood pressure, BMI, and fasting blood glucose of the Subjects also reduced significantly from (141/96 to 121/81), (31.6±0.99 to 24.42 ± 0.67), and (159.5±0.46 to 79.8±1.46), respectively. Levels of biochemical parameters of patients also improved significantly upon repeated administration of *H. umbellata* aqueous fraction. The mechanism of action of *H. umbellata* in the management of Type II diabetes mellitus could be said to be by inhibition of metabolic enzymes.

Type II DM is one of the most common metabolic disorders and a global public health concern associated with lifestyle events or defects. Diabetes is a heterogeneous condition characterized by hyper-glycaemia because of defects in insulin secretion, insulin resistance/action or combination of both of these factors (Ezeja *et al.,* 2015). It is a disease of genetic and environmental origin that affects the glucose-insulin endocrine metabolic regulatory system due to the malfunction of the insulin producing cells of the pancreas. Worldwide estimated prevalence of diabetes among adults in 2010 was 285 million and this value is predicted to rise to 439 million by 2030 (Ozougwu *et al.,* 2013; Okolafor and Ekhaise, 2021). According to Janaka and Luigi (2016), major manifestations of diabetes include: dehydration, excessive urination, and body weakness. Studies have shown that plant extracts have high potential in treating DM. *Hunteria umbellata* (Abeere) (family: Apocynaceae), is highly valued in African traditional medicine (ATM) in the treatment of various human diseases, including of pains, gastric ulcers, liver diseases diabetes mellitus and obesity (Adeneye, 2011). Abeere is one of the herbal remedies that have been used in Nigeria and elsewhere as anti-diabetic therapy. It has demonstrated excellent improvement in DM symptoms in rats studies as hypoglycaemic, hypolipidemic, antioxidants, anti-hypertensive (Adeneye, Adeyemi and Agbaji, 2010; Okolafor and Ekhaise, 2021); fertility treatment and sedative (Adeneye, Olagunju and Murtala, 2019). Several studies have shown *HU* to be an important plant with several health benefits. The plant has been found to have remarkable healing properties, especially in cardiovascular conditions including obesity, hypertension, and diabetes (Ajibola *et al.,* 2018; Fadahunsi *et al.,* 2021). Other important therapeutic benefits of *HU* include healing of the body from liver problems, worm infestation, leprosy, sores, fever, and erectile dysfunction (Ajibola *et al.,* 2017; Oboh *et al.,* 2019**).** Similar results have been reported in human studies with improvements in other bodily functions. Accordingly, existing phytochemicals from the seed, *HU* has the highest fitness score of −11.3 kcal/Mol and acted as a potent anti-diabetic substance with better antibacterial properties in humans (Ladokun *et al*, 2018; Udinyiwe and Aghedo, 2022).

The attending side effects associated with anti-diabetes drugs are enormous. As such, the continuous need to search for a safer natural alternative like those of African traditional medicine (ATM), among which is HU is necessary. While *HU* has been used widely in Nigeria and other places to treat diabetes as an herbal remedy with promising results (Ogunlana *et al.,* 2021), the mechanism of its action in diabetic individuals is yet to be scientifically established. The pharmacological and pharmacodynamics of *HU* on diabetes are yet to be fully established (Ajibola *et al.,* 2018; Aguwamba, 2022). Hence, there is a need to evaluate its phytoconstituents and mechanism of action. Therefore, this research evaluated the phytoconstituents and mechanism of action of Hunteria Umbellata in the Management of Type II Diabetes Mellitus.

MATERIALS AND METHODS

This study employed a double-blinded RCT using 42 participants with type 2 DM. This is to evaluate the effect of HU on individuals with the disease; that is, improving the symptoms of the disease as a primary outcome. The study tested if *HU* exerts a positive effect on other organs such as the liver, kidney, and blood parameters as a secondary outcome. Additionally, toxicology on these organs were observed.

Study Setting: A good part of the study was carried out at the trado-medical clinics in Ibadan; Oyo State, Nigeria on individuals who satisfies the inclusion criteria. Ibadan is the third largest city in Nigeria by population only after Lagos and Kano. It is also the most populous and the capital of Oyo State of Nigeria with a total population of 3, 649,000 as of 2021 (Wikivoyage.org, 2022). Ibadan is the home of the Nigeria's first University; The University of Ibadan, which this study seeks its ethics permission from. The prevalence of *DM* in Ibadan was last studied in 2012 and was 4.7% of the study population mainly healthy civil servants (Ojewale and Adejumo, 2012). Another study was later carried out in a neighbouring town of Oke-Ogun for all categories of individuals with a prevalence of 4.6% (Rasaki *et al,* 2017) indicating a high prevalence of the disease in the state, thus gearing towards an impending diabetes epidemic if measures are not taken. Ibadan offers a verse array of registered

trado-medical clinics, working under an umbrella and providing services to individuals patronising herbal medicines. While it is the largest city, it has over six million inhabitants in its metropolitan area with a consistent growth rate of 2.93% in 2022. Additionally, the demography of the city is not evenly distributed in terms of age; the deprived areas are skewed toward the population of those within 15-64years old when compared to the affluent areas; these are individuals with increased long-term illnesses including DM that also patronise the trado-medical practitioners. This study supports the treatment of DM by evaluating the effect of HU on type two DM. This will subsequently be used to identify those who might benefit from a less expensive and readily available treatment of DM. Finally, the researchers are local as a consideration of negotiating access to the study.

Study Design Randomisation: A double-blinded randomised fraction-controlled trial (RCT) will be used for this investigation. The trial will be conducted in which participants who agree to participate in the study will be randomly assigned to take either H. umbellata fraction or placebo. Participant enrolment and intervention assignment will be arranged by the researchers. The fraction and the placebo will be unidentified to participants, researchers, and investigators. An investigator, who will not be involved in this study, will be engaged to perform simple randomization through a random number list. Forty-two (42) subjects will be assigned with a random numerical code in a 1:1 distribution to one of the two treatment groups. This is to ensure that all communication and correspondence between these codes and the group characteristics is not identified by the researchers and participants.

Blinding: Double blinding was undertaken for the researchers and investigators as well as the subjects for this RCT. Thus, the forty-two (42) subjects were assigned with a random numerical code and also assigned to the containers with the treatments. Both subjects and investigators involved in the trial were blinded to the treatment assignment. Blinding was broken after final results have been obtained and analysed.

Study population: Individuals aged 18 years and above, diagnosed with type II DM registered at trado-medical clinics in Ibadan, Oyo State, Nigeria who satisfies the inclusion criteria were invited to participate in the study. The age restriction is because type II DM develops in adulthood. Additionally, informed consent is sought; hence, the age restriction. Furthermore, the restriction to trado-medical patients is an indication of voluntariness, lack of coercion and willingness of participants.

Inclusion and Exclusion Criteria: Subjects were eligible if they met the American Diabetes Association (ADA) criteria of fasting blood glucose ≥126 mg/dL (7.0 mmol/L) for diabetes (Care and Supplement, 2021). Before studies, biometrics (age, weight, height, BMI, etc.) and lifestyle profiles of the subjects from age 18 years and above would be taken. Subjects with known hypersensitivity to *HU*, pregnant women and those receiving treatment with synthetic anti-diabetes drugs were not admitted for the study. Diabetes mellitus was diagnosed based on the ADA criteria (Hare, M.J. and Topliss, D.J. 2022) as follows: Fasting Plasma Glucose (FPG ≥ 126 mg/dl, Oral Glucose Tolerance Test (OGTT) ≥ 200mg/dl, BMI BMI ≥25 kg/m^2. Other inclusion criteria included: (1) only on herbal remedy (2) age of 18 years and above (3) evidence of previous diagnosis (4) no history of chronic diseases such as immunodeficiency, hypertension, pre-gestational diabetes, kidney disease, or liver disease. Exclusion criteria will include: (1) consuming synthetic anti-diabetes drugs such as metformin, acarbose, etc. (2) multiple enrolments for treatment in trado-medical clinics.

Sample Size Determination: The sample size for this study is 42 participants. This is calculated based on the continuous differences in the primary outcome after four weeks of the intervention to detect a large standardised difference (SD) between the intervention and control groups, with a 90% power and margin of error of 5%. The sample size also takes into consideration the prevalence of population proportion of 4.6% and attrition rate, as it accurately reflects the population being studied within the chosen confidence interval (Calculator.net, 2022).

$$ULP: n = \frac{z^2 x \dot{p}(1-\dot{p})}{\varepsilon^2} \quad 1a$$

$$FP: n' = \frac{n}{1 + \frac{z^2 x \dot{p}(1-\dot{p})}{\varepsilon^2 N}} \quad 1b$$

Where: where ULP = unlimited population; FP = finite population; z = the z score (z score for 90% confidence level is 0.645 or 0.65): ε = the margin of error (5%): N = the population size (3, 649,000): $\hat{p}$ = the population proportion (4.6%)

$$n = \frac{1.65^2 \times 0.5(1-0.5)}{0.05^2} = 42$$

Sampling Technique: The ideal sampling technique for RCT depends on the study design as well as the

prevalence of the disease. Probability sampling is more suitable for RCTs as it provides the opportunity for all the cases to be included in the study while implementing the selection into categories (Saunders, 2011); then stratified according to age, gender, locality, etc. If the inclusion criteria are more restrictive and well defined. Additionally, probability sampling is a feature that distinguishes the quantitative from the qualitative approach (Fischer and Guzet, 2022). However, the sampling procedure could be a constraint for the intervention study as few practices signed up for the research with fewer participants. Furthermore, the probability will limit individuals from participating. For instance, if Mr. B is selected and his friend Mr. C is not, Mr. B will decline to participate. Hence, the best sampling method next to the probability sampling is adopted as all cases available are included as consecutive sampling. The limitations in this sampling strategy could be similarity in ethnicity, street and house, and seasonal and terminal trends. However, the sample for this study includes individuals from diverse ethnic and socio-economic conditions.

Collection and Identification of Plant Materials: Healthy seeds of *HU* were purchased from a local market in Ibadan, Oyo State, Nigeria. The seeds were transported to the laboratory where their peelings were washed/removed. Before this, the seeds were identified and authenticated at the Herbarium Section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The voucher specimen will be deposited in the Herbarium and the specimen identification number obtained.

Preparation, Extraction, and Concentration of Hunteria umbellate: Hunteria umbellata seeds were sun-dried and pulverised to powder with a mechanical grinder for proper solvent extraction. The extraction of *H. umbellata* was carried out using maceration method as described by Majekodunmi (2015). Coarse powder of *H. umbellata* seeds were placed in a stoppered container with the solvent (ethanol) at a solute-solvent ratio of 1 to 10. The suspension was allowed to stand at room temperature for 3 days (72 h) with frequent agitation for adequate extraction of phytocomponents on an electric shaker. The marc was pressed, and menstruum strained with a muslin cloth to obtain the liquid. Combined liquids were clarified by filtration through Whatman No.1 filter paper and cotton wool. The clear filtrate was concentrated using a freeze dryer. The concentrated ethanol extract was stored in a desiccator.

Fractionation of Hunteria umbellata Aqueous Extract into Fractions: Fractionation is a technique that depends on the separation of compounds based on differences in the polarity of solvents. Ethanol extract of *HU* was sequentially partitioned with *n*-hexane, ethyl acetate, and methanol as described by Akinpelu *et al.* (2018) and Oriyomi *et al* (2020). Each time, the extract was dissolved in 4 volumes of distilled water and the solution poured into a clean dry separating funnel. Hexane was first added to the aqueous solution, shaken gently in a capped separating funnel, and allowed to stand for about 30 min for it to separate into layers. The separate layers were tapped off into two separate conical flasks after which fractionation was allowed to continue until the organic layer was devoid of colour. The organic phase (less dense) appearred on top while the aqueous phase (dense) was at the bottom of the separating funnel. The aqueous phase was returned to the separating funnel and the process repeated with ethyl acetate and then methanol. Fractions collected were concentrated *in vacuo* at 40^{o}C and later exposed to air to get rid of remaining traces of solvent. The fractions were weighed, labelled, and kept in a desiccator until needed.

Determination of Percentage Yield of Extract in Different Solvents: The percentage yield of extract was obtained using the Equation 2 (Anokwuru *et al.*, 2011):

$$\% \text{ yield} = \frac{W_2 - W_1}{W_0} \times 100 \qquad (2)$$

Where W_2 is the weight of extract and Petri dish, W_1 is the weight of Petri dish alone and Wo is the weight of initial dried leaves.

In vitro α-amylase inhibitory assay: This assay was conducted using the fraction of the sample as described using a modified procedure of Wickramaratne *et al.* (2016). Test sample of 1 ml in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006M Sodium chloride) containing 1 ml of α- amylase (from Aspergillus oryzae) were pre-incubated at 25 °C for 30 min, after which 1 ml of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added after incubation of the reaction mixture at 25 °C for 10 min. The reaction was terminated by adding 1 ml of 3, 5-dinitrosalicylic acid (DNS) colour reagent (1.0 g of DNS, 20 ml of 2 M of NaOH and 30 g of Sodium potassium tartarate in 100 ml of distilled water). The sample test tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was then diluted with 5 mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The generation of reducing sugar was quantified by reduction of 3, 5-dinitrosalicyclic acid to

3-amino-5-nitrosalicyclic acid. Acarbose was used as a positive control. The α-amylase inhibitory activity was calculated as percentage inhibition based on Equation 3:

$$\%\text{Inhibition} = \frac{[(\text{Abs Control} - \text{Abs Fraction})]}{\text{Abs Control}} \times 100 \quad (3)$$

Concentrations of fractions resulting in 50% inhibition of enzyme activity (IC_{50}) were determinedgraphically.

In vitro α-Glucosidase Inhibitory Activity: This assay was undertaken according to the method described by Li *et al.* (2005). The substrate solution, p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 20 µL of α- glucosidase (0.2 U/mL) was pre-incubated with 20 µL of the different concentrations of the fractions for 10 min. Then 20 µL of 10.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37 °C for 15 min and stopped by adding 80 µL of 0.2m of Na2CO3. The α-glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm with microplate reader. Control contained the same reaction mixture and the same volume of phosphate was added instead of sample solution. Acarbose was dissolved in 10% Dimethyl sulfoxide (DMSO) and used as a positive control. The inhibition percentage was calculated using Equation 4 as follow:

$$\%\text{inhibition} = \frac{[\text{Abs Control} - \text{Abs Sample}]}{Abs\ Control} \times 100 \quad (4)$$

The concentrations of fractions resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

Qualitative Determination of Phytoconstituents: Qualitative analyses of the phytoconstituents of the Whole and Peeled Aqueous HU extract and fractions were carried out using standard methods reported by Attaullah *et al.* (2020).

Quantitative Determination of Phytoconstituents: The total phenolic content (TPC) of HU fractions was determined using spectrophotometric method of Singleton *et al.* (1999) and Gulcin *et al.* (2004). The total tannin content (TTC) of the fractions was determined by measuring spectrophotometrically the catechin equivalent of the fractions using the method described by Singh *et al.* (2012). Total flavonoid content (TFC) was determined by measuring the quercetin acid equivalent of the fractions using the method of Sun *et al.* (1999). The atropin equivalent of the fractions was used to determine the total alkaloid content (TAC) of the samples according to the method of Shamsa *et al.* (2008).

Gas chromatography-mass spectrometry analysis of Whole and Peeled Aqueous Fractions of *Hunteria umbellate:* The fractions were subjected to GC-MS analysis carried out at the University of Lagos, Lagos State, Nigeria. The method of Sulaimon *et al.* (2020) as described by Fagbohun *et al.* (2020) was used to identify the biologically active phytoconstituents.

Preparation of sample for GC-MS: Briefly, 1 g of the dried fractions was steeped in 2 ml each of pyridine and N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) separately. The resulting mixtures were vortexed and oven-incubated at 40 ◦C for 30 min, then cooled at 25 ◦C. Thereafter, the mixtures were filtered with a 0.25µm polyvinylidene difluoride (PVDF) membrane syringe-like filter and extracted using sodium sulfate cartridge, then concentrated to 1 ml with TurboVap at 55 ◦C with nitrogen before being subjected to GC-MS analysis.

Instrumentation and analytical method: A Thermo Scientific DSQII single quadrupole GC-MS (Hewlett Packard Agilent gas chromatography fitted with flame ionization detector and 5975 C Series Injector; Model 19091 J- 413:3516.156884, California, USA) was used to analyze the fraction based on the previous method described by Balla *et al.*, 2017. Interpretation of mass spectra from GC-MS analyses was conducted using a standard database (NIST 11, Colorado, USA). Briefly, the injector (10 µl syringe), transfer line and ion source temperature were maintained at 300, 150 and 230 ◦C, respectively. The GC separation was performed with a capillary column-Agilent 19091S-433HP-5MS (length 30 m x internal diameter 0.25 mm x film thickness 0.25 µm) treated with 5% phenyl and 95% dimethylpolysiloxane. The carrier gas was helium (99.999% purity) operated at 1.4 ml/min. The sample was dissolved in acetone at splitless injection (split ratio of 30:1; a split flow of 45.12 ml/min) of an aliquot sample of ethyl acetate fraction (1 µl), the primary GC oven pressure was maintained at 11.604 psi and the temperature kept constant at 35 ◦C for 5 min before being raised by 4 ◦C/min to 150 ◦C for 2 min. The temperature of the secondary oven was also held isothermally at 35 ◦C for 5 min then raised by 20 ◦C/min to 250 ◦C for 5 min. The slow fan was disabled throughout the analysis. The total run time was 45.75 min at a flow rate of 1.5 ml/min. The detector of the qMS was operated in scan mode between 50 and 750 amu and the ion source run at 2647 eMV. The mass spectrum solution software provided by Agilent was used to control the system and acquire the mass spectra. A scan interval of 5 min and a fragment from

50 to 600 Da was maintained. The compounds were identified by comparing the mass spectra (peak) obtained with those of the standard mass spectra obtained from the NIST 11 (National Institute of Standards and Technology) library or database.

Pharmacological intervention and Biochemical Analyses: After randomization involving 42 patients, 21 were assigned to receive *H. umbellata* capsule (500 mg) before breakfast, lunch, and dinner for 28 days according to the method of Ezeja, Anaga and Asuzu, (2015). The remaining 21 patients were administered calcined magnesia capsules (500 mg) as a placebo at the same dose in the same pharmacological presentation for 28 days. All capsules were identical to assure blindness. A treatment compliance ≥80% was acceptable, and it was monitored through a personal diary and medication observation.

Procedures and calculations: Patients were evaluated before and after the pharmacological intervention. All patients received medical nutritional therapy (HU and placebo) and were instructed to continue their normal physical activity. Height and body weight were measured with individuals wearing light clothing and no shoes. Height was measured with subjects standing and the head aligned in when the orbitale is in the same horizontal plane as the tragion. Measurements were rounded to the nearest meter. Body weight was evaluated using a body weight measuring scale and results were reported in kg (1dp). BMI was calculated by dividing body weight (kg) by height squared (m^2). Waist circumference was measured with a flexible, steel, certified, Lufkin tape at the midpoint between the lowest rib and the iliac crest and was expressed in centimeters. Blood pressure was measured on the left arm with the subject seated in a chair after a 5-min rest, with his arm stretched at heart level in a flat surface, and with a digital sphygmomanometer (Omron Hem-907 XL). Measurements of systolic blood pressure and diastolic blood pressure were expressed in mmHg.

Collection of Blood Sample for Biochemical Tests: Blood samplings were performed early in the morning after an overnight fast of 10-12 h. The venipuncture was carried out by a trained and experienced Phlebotomist under suitable conditions. Serum liver enzymes like alanine transaminase (ALT), aspartate transaminase (AST), high-density lipoprotein cholesterol (HDL), triglycerides (TG), creatinine (Cr) were measured using Randox kits (Crumlin, UK). Hemoglobin A1c (HbA1c) levels were assessed by a high-performance liquid chromatography analyzer (Tosoh, Tokyo, Japan). Blood samples were collected into K3-EDTA anticoagulated bottles using a needle and a syringe for individual patients for the estimation of biochemical parameters (Chawla, 1999). Collected blood samples were centrifuged at 4000 rpm for 10 minutes with a Table Centrifuge (Model 90-2, Micro field instrument, Essex England) at 4^{o}C. The plasma was carefully collected into sterile bottles with sterile Pasteur pipettes. The sample bottles containing blood plasma were then labelled and kept at -4^{o}C for biochemical analyses.

Determination of Blood Glucose Level: The initial fasting blood glucose was taken using a glucometer with acute test strip. The weekly blood glucose was taken in all groups of patients by pricking the thumb to allow blood to drop on a strip fastened to the glucometer; this was carried out weekly for the two weeks study (Bain *et al.*, 2017).

*Biochemical Analyses (Liver and Kidney function tests***)**: The liver and kidney function tests were carried out spectrophotometrically on the sera using Randox Diagnostic kits. Collected sera were analyzed for total protein (TP), aspartate transaminase (AST), alanine transaminase (ALT), urea, and creatinine following the methods of Reitman *et al.* (1957) and Azwanida (2015).

Determination of Plasma Lipid Profiles: The serum triglyceride (TG) and HDLcholesterol (HDL-Chol) were determined according to the methods of Friedewald *et al.* (1972) using Randox Diagnostic kits.

Data Analysis: Statistical analysis was carried out using SPSS Version 27 after monitoring and cleaning of the data collected from all the participants. The analysis was undertaken by a Biostatistician and investigators, while considering the mean difference, attrition rate and missing data in the analysis of variance procedure. Data collected were managed through a confidential online folder run through the University of Ibadan; the participants' information files were stored under lock and key at the University of Ibadan.

RESULTS AND DISCUSSION

Descriptive statistics of frequency and percentages were used to summarize the categorical parameters. The parametric distribution for continuous variables were assessed by Shapiro-Wilk normality test. Median and Interquartile range was used to summarize continuous parameters. Pearson's Chi-Squared test and Fisher's exact test were used in the appropriate scenario to show statistical significance between two categorical variables. The Wilcoxon rank sum was used to test for significant differences in median values of two independent continuous variables. The data was analyzed using R Statistical Computing version 4.0.2.

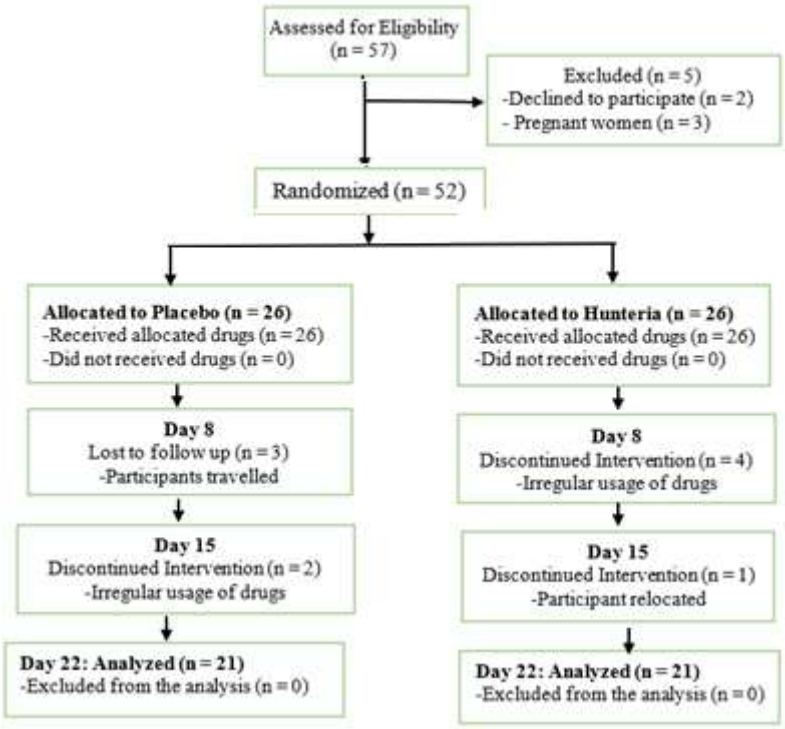

Fig 1: Flow-chart of the study showing participants' progress of intervention.

Table 1. Sociodemographic of the Participants

Variable	N	**Overall**, N = 42[1]	**Type of Drugs**		p-value[2]
			HU, N = 21[1]	**PL**, N = 21[1]	
Age [Years]	42	57 (52, 61)	59 (56, 61)	56 (50, 61)	0.262
Sex	42				0.212
Female		18 (43%)	7 (33%)	11 (52%)	
Male		24 (57%)	14 (67%)	10 (48%)	
Education	42				>0.999
Secondary		26 (62%)	13 (62%)	13 (62%)	
Tertiary		16 (38%)	8 (38%)	8 (38%)	
Occupation	42				0.384
Artisan		6 (14%)	4 (19%)	2 (9.5%)	
Civil Servant		17 (40%)	9 (43%)	8 (38%)	
Farmer		6 (14%)	4 (19%)	2 (9.5%)	
Trader		13 (31%)	4 (19%)	9 (43%)	
Smoking	42	2 (4.8%)	1 (4.8%)	1 (4.8%)	>0.999
Alcohol	42	16 (38%)	9 (43%)	7 (33%)	0.525

[1] Median (IQR) or Frequency (%)
[2] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

A total of 57 individuals were assessed for eligibility and 5 were excluded as they did not meet the inclusion criteria (pregnancy and declined to participate). Fifty-two participants were randomly selected into one of the two groups: Placebo (PL) and Hunteria (HU), and the baseline measures were assessed after randomization. During the 21-day intervention, ten participants (Placebo group, n = 5; Hunteria group, n = 5) dropped out due to travelling, relocation, personal scheduling conflicts, and irregularities in the usage of drugs. The remaining 42 participants completed the trial and were included in the analysis.

Table 1 shows the demographic distribution of the subjects that participated in the study. The table clearly revealed that the subjects were randomly chosen. However, the choice of subject with minimum of secondary school education was deliberate. This is to ensure that the subjects have very good understanding of the terms of the study. Many of the potential subjects without formal education were either scary or associated the study to ploy by government to force tax evaders to pay tax. The distribution by occupation shows that the study cut across all the strata of human endeavours.

Table 2. Medical History of the Participants

Parameter	N	Overall, N = 42[1]	Type of Drugs		p-value[2]
			HU, N = 21[1]	PL, N = 21[1]	
Erectile Dysfunction	42	16 (38%)	13 (62%)	3 (14%)	0.001
Rheumatism	42	6 (14%)	5 (24%)	1 (4.8%)	0.184
Bedtime Urination	42	20 (48%)	8 (38%)	12 (57%)	0.217
Bedtime Urination	42				0.09
0		22 (52%)	13 (62%)	9 (43%)	
1		8 (19%)	1 (4.8%)	7 (33%)	
2		11 (26%)	6 (29%)	5 (24%)	
3		1 (2.4%)	1 (4.8%)	0 (0%)	
Age Diagnosed	42	54 (49, 58)	57 (52, 59)	52 (45, 55)	0.005

[1] Median (IQR) or Frequency (%)
[2] Pearson's Chi-squared test; Fisher's exact test; Wilcoxon rank sum test

In Table 2, the stochastic distribution has been based on biometry. The analysis contained in Table 2 is based on data collected from the physiological manifestation of the biological phenomena that have been associated with diabetes. The table clearly emanated from the symptoms of the ailment. In the same vein, Table 3 is a measure of physical fitness of an individual using basic parameters such as overweight, obesity, and other physical parameters as a cautionary measure for patients who are prone to diabetes.

Table 3. Baseline Fasting Blood Glucose, Blood Pressure and Biometric

Variable	N	Overall, N = 42[1]	Type of Drugs		p-value[2]
			HU, N = 21[1]	PL, N = 21[1]	
Height [m]	42	1.67 (1.61, 1.70)	1.67 (1.65, 1.69)	1.67 (1.58, 1.72)	0.909
Weight [Kg]	42	80 (74, 88)	80 (74, 88)	79 (76, 87)	0.9
BMI [Kg/m^2]	42	30.2 (27.2, 32.8)	29.8 (27.2, 31.6)	30.3 (27.6, 33.3)	0.86
Waist Circumference [cm]	42	91.4 (86.4, 96.5)	91.4 (86.4, 96.5)	94.0 (88.9, 96.5)	0.46
Hip Circumference [cm]	42	96.5 (91.4, 104.1)	94.0 (91.4, 99.1)	99.1 (91.4, 104.1)	0.376
Waist-Hip Ratio	42	0.953 (0.942, 0.972)	0.971 (0.944, 0.973)	0.949 (0.936, 0.972)	0.319
Diastolic	42	90 (80, 104)	90 (72, 105)	92 (85, 101)	0.83
Systolic	42	132 (126, 140)	140 (132, 150)	128 (120, 131)	<0.001
Baseline FBG [mg/dL]	42	165 (153, 178)	168 (162, 184)	162 (148, 173)	0.085

[1] Median (IQR) or Frequency (%)
[2] Wilcoxon rank sum test

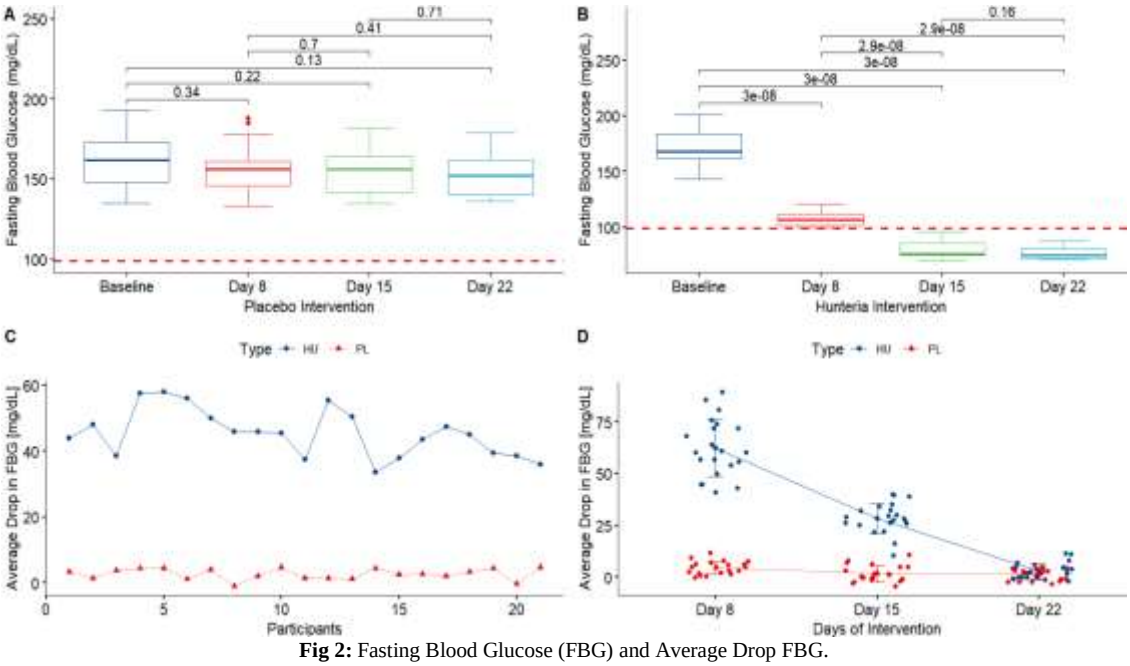

Fig 2: Fasting Blood Glucose (FBG) and Average Drop FBG.

Figure 1A is a comparison of FBG level between baseline and days of placebo intervention. Median (IQR) for Day 8, Day 15, and Day 22 were 156 (146 – 161), 156 (142 – 164), and 152 (140 – 162) mg/dL respectively. There is no sufficient statistical evidence of differences in FBG Level. None of the participants FBG level drop was below 99 mg/dL throughout the 21 days of Placebo experiment. Figure 1B compares FBG level between baseline and days of Hunteria Intervention. The Day 8, Day 15 and Day 22 values were 107 (102 – 112), 76 (75 – 86), and 75 (72 – 81) mg/dL respectively. There were statistically significant differences in FBG between baseline and (Day 8, Day 15, Day 22) in Hunteria Intervention (all p-value < 0.001). There were also substantial statistical differences between Day 8 and Day 15; Day 8 and Day 22 (all p-value < 0.001). There was no significant difference between FBG levels between Day 15 and Day 22 (p-value = 0.16). It was observed that Day15 and Day 22 FBG were below 99 mg/dL for all participants in Hunteria cohort. Figure 1C contains the profile of the average drop in FBG per participants during the period of the trial. It revealed an average drop in FBG of 45.5 mg/dL [mean (SD) of 45.5 (7.1) mg/dL] for patients treated with Hu, while average drop in FBG was 2.61 mg/dL [2.61 (1.65) mg/dL] for placebo. Figure 1D is a plot of average drop in FBG on Day 8, Day 15, and Day 22. On Day 8 the drop in FBG was the difference between FBG of Baseline and FBG of Day 8; Day 15 FBG drop was difference between Day 8 FBG and Day 15 FBG; Day 22 FBG drop was difference in Day 15 FBG and Day 22 FBG. Day has the mean (SD) of PL and HU to be 4.57 (3.02) and 62.43 (13.67) respectively. Similarly, Day 15 and Day 22 average drop in FBG are [PL = 1.76 (3.84), HU = 28.48 (7.10)] and [PL= 1.38 (2.68), HU = 3.05 (3.67)] respectively.

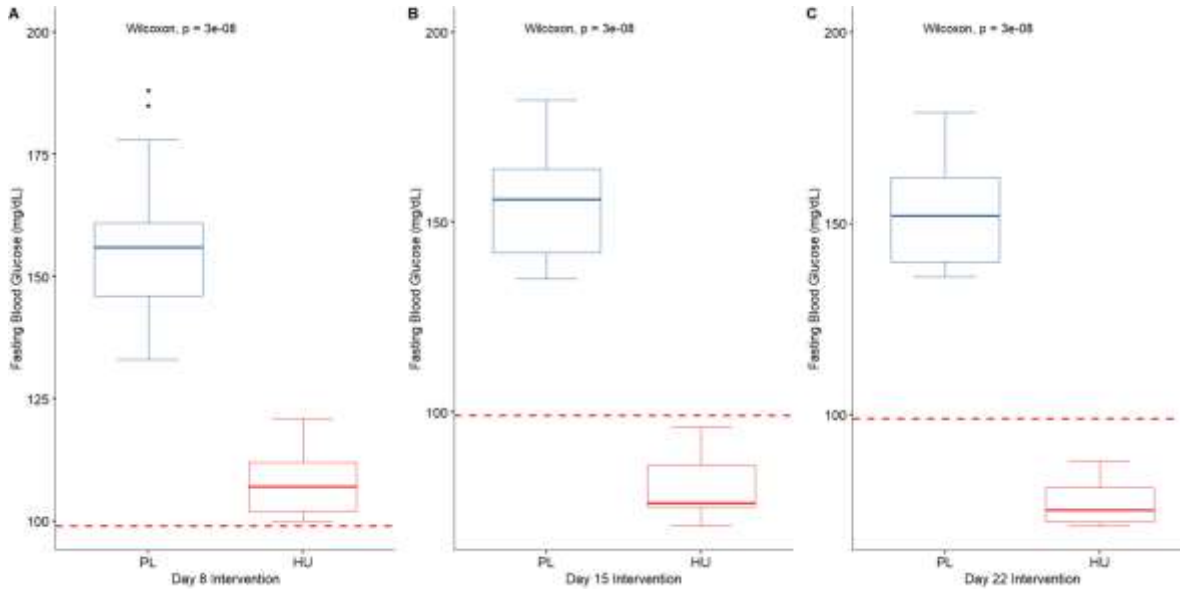

Fig 2: Comparison of FBG between PL and HU in Day 8, Day 15, and Day 22.

Figure 2A: Day 8 Comparison of FBG Level between PL and HU. There was statistical difference between PL and HU interventions FBG (p < 0.001). However, FBG Level in both interventions is more than 99 mg/dL. Figure 2B: Day 15 Comparison of FBG Level between PL and HU. There was statistically significant difference between PL and HU interventions FBG (p < 0.001). On Day 15 HU intervention FBG Level was below 99 mg/dL while PL intervention FBG is above 99 mg/dL. Fig 1C: Day 22 Comparison of FBG Level between PL and HU. There was statistically significant difference between PL and HU interventions FBG (p < 0.001). PL FBG Level is more than 99 mg/dL while HU FBG Level is less than 99 mg/dL. Figure 3A shows that there was an average drop in FBG stratified by Sex. There was substantial difference of average drop of FBG in Female compared to that of Male subjects (p = 0.033). Figure 3B reveals the facts about whether there was an average drop in FBG by Age Group. There was no statistical difference of FBG in the age group (all p > 0.05). Figure 3C was aimed at making a statement about the effect of waist ratio on the manifestation of diabetes by showing whether there exists an average drop in FBG stratified by Waist-Hip Ratio (WHR). The average drop of FBG in Normal and Risk WHR was not significant. Figure 3D dwelt on average drop in FBG grouped by BMI category. No difference between overweight and obese BMI average drop of FBG (p = 0.11). Similarly, Figure 3E, Figure 3F, and Figure 3G are the average drop in

FBG stratified by Age after diagnosed with diabetes, physical activities, and nocturia (urination of more than once during night sleep**).** All showed no significant difference (all $p > 0.05$). Figure 3H: The Relationship between Average Drop in FBG and Blood Pressure. It was observed that there was a significant positive linear association between average drop in FBG Level and Systolic blood pressure ($r = 0.44$, $p = 0.043$).

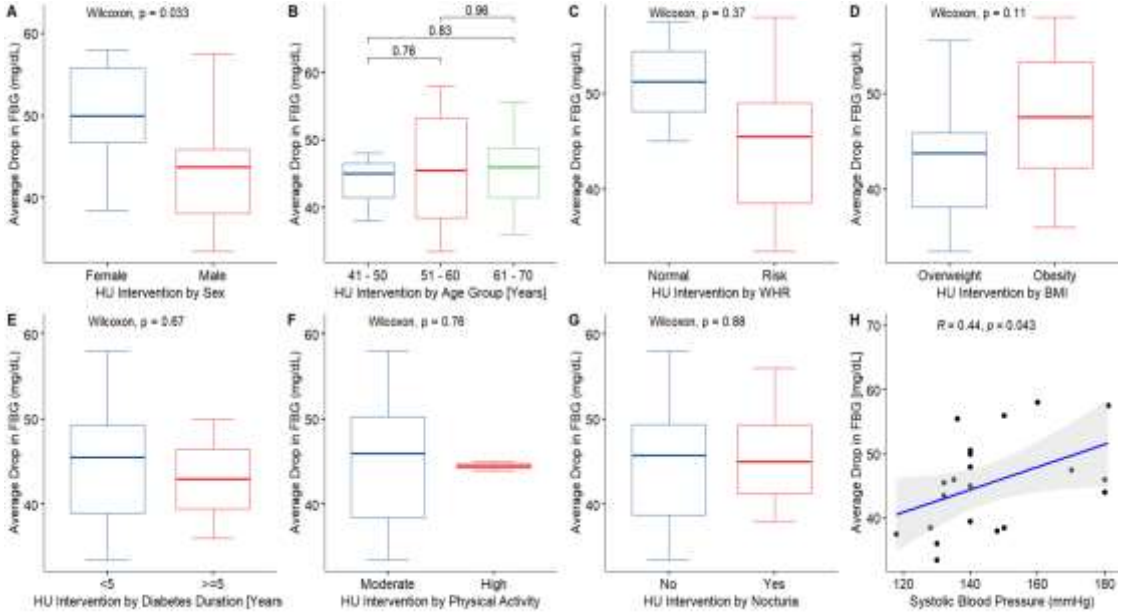

Fig 3: The Effect of HU on Parameters.

Conclusion: This RCT demonstrates that HU could be used as a potent treatment of type II diabetes mellitus. The study showed that normal fasting blood glucose is restored within fifteen days of administering 500 mg of Hunteria Umbellata thrice daily before or after meal. There is also a significant effect of HU on FBG in female than the male subjects, indicating an even more suitability for the female subjects. We advocate for more research using diverse samples in different locations for generalisation.

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